TRYPSIN DIGESTING MEDIUM ADDED WITH HORSE PLASMA FOR THE CULTURE OF THE GONOCOCCUS*

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To identify *Neisseria*-like colonies suspected to be gonococci, it is necessary to use the " oxydase " reaction of Gordon and McLeod (1928) ; colonies sprayed with a 1 per cent. solution of dimethyl paraphenylene hydrochloride turn pink *immediately* on contact with the oxidizing fluid, and then black in 2 to 3 minutes. If blood media are used, the " oxydase " reaction is not readily visible owing to their dark colour. The use of tri- or tetramethyl paraphenylene simplifies the problem, and efficient sub-cultures are possible, but these chemicals are scarce and expensive.

Since 1948, at the Hôpital St. Lazare, where the culture of the gonococcus is our routine practice, 8,000 cultures (from both sexes) have been done, including 1,883 from prostitutes, with 818 positive results, *i.e.* 43·5 per cent. The following media were used in successive experiments :

(1) Peptone medium with fresh human blood (Roiron-Ratner, 1949) ;

(2) Cooper and McLeod's heated horse-blood medium, (Cooper and others, 1950 ; Wilkinson, 1952) ;

(3) Horse haemoglobin medium of Peizer and Steffen (1942) modified by Le Minor (1948).

We attempted to devise a colourless medium which would facilitate the oxidation process and at the same time allow subcultures from these colonies as the bacteria thereon remain alive in spite of this treatment. As a result of investigation the use of the medium described below was adopted.

Constitution and Method of Preparation

(A) To 100 g. defatted minced beef mixed with 500 ml. glass-distilled water add 15 ml. N/1 NaOH and heat for 5 minutes at 75–80° C. After cooling to 40° C., add 0·2 g. powdered trypsin and incubate the whole mixture at 37–38° C. for 6 hrs. After this period, stop the trypsin digestion with 0·7 ml. glacial acetic acid and 10 minutes boiling. Filter through paper, adjust to *p*H 7·6–7·8 with N/1 NaOH.

(B) Take 12·5 g. agar-agar previously washed in tap-water and dissolve in a 1 per cent. sodium chloride solution.

(C) Mix together equal parts of A and B. Add 1 g. glucose and 5 g. $NaHPO_4$.

The mixture is then poured into flasks or tubes and autoclaved (110° C. for 20 minutes).

For use, a flask of the agar broth is taken out and the content is melted over a water-bath ; after cooling to 50–60° C., add 20 per cent. citrated horse plasma and 500 u. penicillinase. Mix well and pour into Petri dishes. The plasma must be sterile or filtered through Seitz pads.

The stock medium without plasma can be stored for one month at laboratory temperature and the whole medium for 8 days at + 4° C.

Advantages of this Medium

In the beginning we used this medium together with those previously mentioned, and obtained the same percentage of positive results in growth of gonococci. But with the use of the *new medium, which has only a pale yellow colour, the advantages lie in the ability to watch the " oxydase " reaction from the beginning, and to pick out colonies more easily for sub-cultures* on sugar media to complete the identification.

This identification is in fact essential, owing to the presence in the genital tract of *Neisseria* other than gonococci. Some figures are quoted below :

Wax (1949) had a particularly high percentage : out of 442 strains studied by him, 19 per cent. were non-gonococcal.

Wilkinson (1952) found these pseudo-gonococcus *Neisseria* in 3·4 per cent. of women and 1·5 per cent. of men out of 1,575 cultures investigated.

We found non-glucose fermenting *Neisseria* in many instances, particularly in women during the test for cure.

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In addition, the new medium proved to be inexpensive because it does not contain any commercial peptone, this being replaced by predigested meat (trypsin), a procedure frequently used in the U.S.A., especially in the culture medium advocated by Dienes (1939) for the isolation of PPLO. It was actually while handling this medium that we thought of its possible utilization for the gonococcus culture, adding dibasic sodium phosphate for the "oxydase" reaction, and penicillinase so that culture would be possible even in the midst of a course of penicillin treatment.

We replaced the Difco agar-agar by a French one in sufficient concentration to obtain a *firm* though *not too hard* medium, this being of prime importance for the satisfactory growth of the gonococcus.

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